

**Amendments to the Claims:**

*This listing of claims will replace all prior versions and listings of claims in the application.*

**Listing of Claims:**

1. (Withdrawn-Currently Amended) An assay for determining the level of prostacyclin in plasma comprising:

- (1) providing a plasma sample;
- (2) incubating the plasma sample with an effective amount of ~~an anti-6-keto-PGF<sub>1 $\alpha$</sub>~~   
~~primary antibody, a secondary antibody and 6-keto-PGF<sub>1 $\alpha$</sub> -aequerin conjugate;~~  
an anti-6-keto-prostaglandin F<sub>1 $\alpha$</sub>  (6-keto-PGF<sub>1 $\alpha$</sub> ) antibody; an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF<sub>1 $\alpha$</sub> -antibody; and a conjugate comprising 6-keto-PGF<sub>1 $\alpha$</sub>  covalently bound to an aequerin mutant;

wherein said aequerin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequerin (Cys  $\rightarrow$  Ser), wherein said aequerin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69  $\rightarrow$  Cys), 70 (Gly70  $\rightarrow$  Cys), 74 (Gly74  $\rightarrow$  Cys) or (Glu76  $\rightarrow$  Cys), and

wherein the 6-keto-PGF<sub>1 $\alpha$</sub>  is coupled to the aequerin mutant via reaction with the sulfhydryl group of the single cysteine;

- (2) (3) removing any unbound ~~primary anti-6-keto-PGF<sub>1 $\alpha$</sub> -antibody and said conjugate 6-keto-PGF<sub>1 $\alpha$</sub> -aequerin conjugate~~ from the plasma sample following incubation; and
- (3) (4) measuring and correlating light intensity of the plasma sample with amount of prostacyclin within the plasma sample.

2. (Withdrawn-Currently Amended) The assay of claim 1 wherein the ~~secondary anti-immunoglobulin antibody that binds to the anti-6-keto-PGF<sub>1 $\alpha$</sub> -antibody~~ antibody is coated onto a surface which is exposed to the plasma, ~~primary antibody anti-6-keto-PGF<sub>1 $\alpha$</sub> -antibody and said 6-keto-PGF<sub>1 $\alpha$</sub>  conjugate.~~

3. (Cancelled).

4. (Withdrawn) The assay of claim 1 wherein the plasma sample is obtained from a patient receiving intravenous prostaglandin therapy.

5. (Withdrawn-Currently Amended) The assay of claim 1 wherein the concentration of said conjugate 6-keto-PGF<sub>1 $\alpha$</sub> -aequorin-conjugate in the assay is about  $1 \times 10^{-10}$  M.

6. (Cancelled).

7. (Cancelled).

8. (Withdrawn-Currently Amended) A method of determining an appropriate dose of prostaglandin for the treatment of primary pulmonary hypertension in a patient comprising

(1) providing a plasma sample from the patient;

(2) incubating the plasma sample with an effective amount of ~~anti-6-keto-PGF<sub>1 $\alpha$</sub> -primary antibody, a secondary antibody and 6-keto-PGF<sub>1 $\alpha$</sub> -aequorin-conjugate;~~

an anti-6-keto-prostaglandin F<sub>1 $\alpha$</sub>  (6-keto-PGF<sub>1 $\alpha$</sub> ) antibody; an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF<sub>1 $\alpha$</sub> -antibody; and a conjugate comprising 6-keto-PGF<sub>1 $\alpha$</sub>  covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys  $\rightarrow$  Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69  $\rightarrow$  Cys), 70 (Gly70  $\rightarrow$  Cys), 74 (Gly74  $\rightarrow$  Cys) or (Glu76  $\rightarrow$  Cys), and

wherein the 6-keto-PGF<sub>1 $\alpha$</sub>  is coupled to the aequorin mutant via reaction with the sulphydryl group of the single cysteine,

(3) removing any unbound ~~primary~~ anti-6-keto-PGF<sub>1 $\alpha$</sub> -antibody and said conjugate from the plasma sample following incubation;

(4) measuring and correlating the amount of detected 6-keto-PGF<sub>1 $\alpha$</sub>  with the appropriate dosage of prostaglandin for the patient.

9. (Withdrawn-Currently Amended) The method of claim 8 wherein the ~~secondary antibody~~ anti-immunoglobulin antibody is coated onto a surface which is exposed to the plasma, ~~primary anti-6-keto-PGF<sub>1α</sub>-~~ antibody and ~~6-keto-PGF<sub>1α</sub>-aequerin~~ said conjugate.

10. (Cancelled).

11. (Withdrawn) The assay of claim 8 wherein the plasma sample is obtained from a patient receiving intravenous prostaglandin therapy.

12. (Withdrawn-Currently Amended) The assay of claim 8 wherein the concentration of ~~6-keto-PGF<sub>1α</sub>-aequerin~~ said conjugate in the assay is about  $1 \times 10^{-10}$  M.

13. (Withdrawn-Currently Amended) An assay for determining the level of a biomolecule in plasma comprising:

(1) providing a plasma sample;

(2) incubating the plasma sample with an effective amount of a ~~primary antibody~~ an anti-6-keto-prostaglandin F<sub>1α</sub> (6-keto-PGF<sub>1α</sub>) antibody to the biomolecule, a ~~secondary antibody~~ an anti-immunoglobulin antibody that binds to the biomolecule and a biomolecule-aequerin conjugate comprising 6-keto-PGF<sub>1α</sub> covalently bound to an aequerin mutant;

wherein said aequerin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequerin (Cys → Ser), wherein said aequerin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69 → Cys), 70 (Gly70 → Cys), 74 (Gly74 → Cys) or (Glu76 → Cys), and

wherein the 6-keto-PGF<sub>1α</sub> is coupled to the aequerin mutant via reaction with the sulfhydryl group of the single cysteine;

(2) (3) removing any unbound primary-antibody anti-6-keto-PGF<sub>1α</sub> antibody and biomolecule-aequerin conjugate from the plasma sample following incubation; and

(3) (4) measuring and correlating light intensity of the plasma sample with amount of biomolecule within the plasma sample.

14. (Withdrawn-Currently Amended) The assay of claim 13 wherein the ~~secondary antibody anti-immunoglobulin antibody~~ is coated onto a surface which is exposed to the plasma, ~~primary antibody anti-6-keto-PGF<sub>1α</sub> antibody~~ and biomolecule-aequorin conjugate.

15-18. (Cancelled).

19. (Withdrawn): The biomolecule aequorin conjugate of claim 17 wherein the biomolecule is a peptide.

20. (Withdrawn-Currently Amended) A method for determining the effect of a therapeutic agent on the level of prostacyclin in the plasma of a patient comprising

- (1) administering the therapeutic agent to the patient;
- (2) obtaining a plasma sample from the patient;
- (3) incubating the plasma sample with an effective amount of ~~anti-6-keto-PGF<sub>1α</sub> primary antibody, a secondary antibody and 6-keto-PGF<sub>1α</sub>-aequorin conjugate;~~  
an anti-6-keto-prostaglandin F<sub>1α</sub> (6-keto-PGF<sub>1α</sub>) antibody; an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF<sub>1α</sub>-antibody; and a conjugate comprising 6-keto-PGF<sub>1α</sub> covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys → Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69 → Cys), 70 (Gly70 → Cys), 74 (Gly74 → Cys) or (Glu76 → Cys), and

wherein the 6-keto-PGF<sub>1α</sub> is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine;

- (4) removing any unbound ~~primary antibody anti-6-keto-PGF<sub>1α</sub> antibody~~ and 6-keto-PGF<sub>1α</sub>-aequorin ~~said~~ conjugate from the plasma sample following incubation; and

- (5) measuring and correlating light intensity of the plasma sample with amount of prostacyclin within the plasma sample.

21. (Cancelled).

22. (New) A kit for measuring prostacyclin in plasma comprising:

- (1) an anti-6-keto-prostaglandin  $F_{1\alpha}$  (6-keto-PGF $_{1\alpha}$ ) antibody;
- (2) an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF $_{1\alpha}$ -antibody; and

- (3) a conjugate comprising 6-keto-PGF $_{1\alpha}$  covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys  $\rightarrow$  Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69  $\rightarrow$  Cys), 70 (Gly70  $\rightarrow$  Cys), 74 (Gly74  $\rightarrow$  Cys) or (Glu76  $\rightarrow$  Cys), and wherein the 6-keto-PGF $_{1\alpha}$  is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine.